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# THE EFFECT OF PEROXIDE OXIDATION OF MICROSOMAL LIPIDS ON THE SPECTRAL CHARACTERISTICS OF CYTOCHROME P-450

V. V. Lyakhovich, I. B. Tsyrlov, V. M. Mishin
and O. A. Gromova

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16. Abstract

The authors studied the influence of activation of both enzymatic and non-enzymatic lipid peroxidation systems in the rat liver microsomal fraction on the spectral properties of cytochrome P-450 and on the hydrophobity of microsomal membranes. It has been shown that in the course of both types of peroxidation, the degree of membranous hydrophobity greatly decreases, and as a result of this cytochrome P-450 is transformed into its inactive form -- cytochrome P-420. The conversion of cytochrome P-450 into P-420 and the decrease in ANS-fluorescence intensity were completely prevented in the presence of both EDTA and phenergan which simultaneously remove the lipoperoxidative effect.

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# THE EFFECT OF PEROXIDE OXIDATION OF MICROSOMAL LIPIDS ON THE SPECTRAL CHARACTERISTICS OF CYTOCHROME P-450

V. V. Lyakhovich, I. B. Tsyrlov, V. M. Mishin and O. A. Gromova[1]

The unusual lability of cytochrome P-450 is explained by the hydrophobe surrounding of heme in the membrane of the endoplasmatic reticulum, which is provided for both by a certain conformation of the hemoprotein itself and by the lipid component of the membrane [1]. /897*

The conversion of P-450 into the enzymatically inactive form P-420, brought about by solubilizing agents which attack the phospholipids can indicate that the microsomal phospholipids are responsible for the spectral properties of the cytochrome [2, 3].

This paper is a study of the interaction between a change in the hydrophobity of the microsomal membrane (as the result of activation in it of POL reactions) and the spectral characteristics of cytochrome P-450, as well as the influence of EDTA and the antioxidant phenergan (pypolphen) on these processes. The change in the hydrophobity was caused by NADPH-dependent and non-enzymatic systems of peroxide oxidation of unsaturated aliphatic acids (UAA). The preliminary results were published earlier by us [4].

## The Methods of the Investigation

In the experiments male rats of the Wistar strain were used; these rats varied in weight from 150-180 grams. The fraction of microsomes was obtained by differential centrifuging [5] in a medium consisting of 0.35 M saccharose, 0.025 M KCl, 0.01 M $MgCl_2$, 0.05 M tri-HCl, pH 7.5. Subsequent centrifuging was carried out in the VAC-60 ultracentrifuge at 105,000 g for a period of

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[1]Central Scientific Research Laboratory of the Novosibirsk State Medical Institute.

*Numbers in the margin indicate pagination in the foreign text.

60 minutes. The precipitate of particles was suspended in 125 mM KCl + 20 mM tri-HCl, pH 7.4 such that 1 ml of suspension contained 23-30 mg microsomal protein.

The activity of the systems of peroxide oxidation of unsaturated aliphatic acids in the microsomal membranes was determined by the amount of formed malonic dialdehyde (MDA) by the aid of the thiobarbituric reaction [6], using the molar coefficient of extinction $\varepsilon^{1\ cm}_{535\ nm} = 1.56 \cdot 10^5$ [7]. Under our conditions, after 10 minutes of nonenzymatic peroxide oxidation 37.5 nm MDA/mg protein was measured, while over the same period of time, with startup of the enzymatic peroxide oxidation, 48 nm MDA/mg protein was determined (average of 9 runs).

The composition of the incubated mixture of NADPH-dependent and ascorbate--dependent systems of POL was identical to that used by the authors [6], with the addition of the ADP + $Fe^{3+}$ complex as described in the work [8] for achieving the maximum rate of lipoperoxidase reactions.

The time of incubation was calculated from the moment of adding 0.8 mM NADPH, while all parameters to the point of adding NADPH to the medium were estimated as indices of "zero time". In the same fashion, after recording the differential spectromicrosomal carriers at zero time, the original OP suspension of microsomes (taken as 100%), and also the intensity of fluorescence of $ANS^-$, by adding 1 mM ascorbate to the cuvette, the reaction of nonenzymatic peroxide oxidation of the microsomal lipids was initiated. The time of incubation was 10 minutes in the ultrathermostat at 37° with continual agitation of the probe. At the end of the period of incubation the reaction was stopped by adding 300 mkM EDTA. In necessary cases incubation was carried out in the presence of EDTA or 5 μM phenergan. The details of the tests are cited in the Figure captions. /898

The absorption of oxygen was recorded using the LP-7 polarograph with standard platinum electrode. The change in optical density of the microsomal suspension over the course of incubation was measured spectrophotometrically at 520 nm [9]. The intensity of fluorescence of $ANS^-$ (8-aniline-1-naphthalen sulfonic acid) in the cuvette after adding microsomal suspension was recorded on the spectrofluorimeter using the paper recording method. The concentration of ANS was 10 μM.

The qualitative determination of the content of microsomal cytochromes $b_5$ and P-450 was determined in accordance with a method described by Omura and Sato [2, 3] on the "Hitachi" model 356 differential double beam recording spectrophotometer. The coefficients of millimolar extinction are: for P-450 -- 91 $mM^{-1}$ $cm^{-1}$ with a difference in absorption at 450 and 490 nm, for P-420 -- 111 $mM^{-1}$ $cm^{-1}$ ($\Delta\Omega\Pi$ 420-490 nm) and for $b_5$ -- 163 $mM^{-1}$ $cm^{-1}$ with a difference in absorption spectra at 424 and 409 nm.

The content of microsomal protein was determined by the Lowry method [10].

Results and Their Discussion

From Figure 1, one can see that the course of reactions of POL in the microsomal suspension are induced both by NADPH + ADP-$Fe^{3+}$, and by ascorbate + + ADP-$Fe^{3+}$, accompanied by a drop in the absorption peak at 450 nm and by the appearance of a significant extinction at 420 nm. In this regard it is important to emphasize the following. In the first place, one notes an absence of stoichiometry between the spectrally recorded decrease in P-450 and the increment in P-420, which has also been noted by other authors when processing the microsome with n-chlormercuribenzoate (n-ChMB) [11], or with solubilizing agents [2, 3]. In the second place, a comparison of the data in Figure 1 with the values of the accumulation of MDA for each reaction (see the table) makes it /899 possible to suggest that it is in fact the degree of decrease in P-450 (but not the increase in P-420) which actually reflects the weight of inactivation of hemoprotein in the investigated POL reactions.

In discussing the reason for the spectral changes in cytochrome P-450 recorded by us, one can pose the question as to whether or not the decrease in P-450 and the appearance of P-420 with a change in the hydrophobity of the microsomal membrane itself are related, or whether or not the detected spectral transitions were caused by the indirect action of products of peroxide oxidation. The drop in optical density of the microsomal suspension detected by us at 520 nm (see table) indicates, according to the data of Tam and McCay, damage to the microsomal structure as the result of damage to the phospholipid component of the microsomal membrane [9]. It has been shown, on the other hand [12], that adding the hydroperoxides of unsaturated aliphatic acids or MDA to the microsomal suspension does not lead to a decrease in the activity of

glucose-6-phosphatase of the POL microsomal enzyme most sensitive to activation. Hence, it is logical that the reason or this conversion is a decrease in the hydrophobity of the microsomal membrane which occurs under our conditions as the result of deterioration of the phospholipid component.

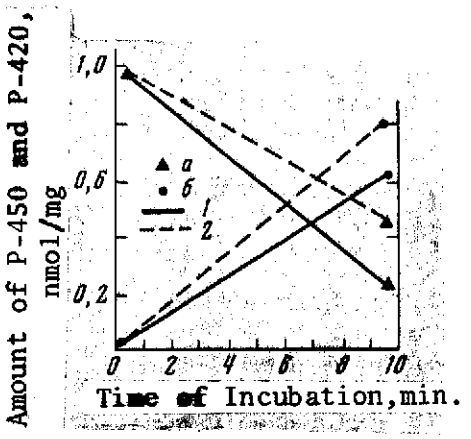


Figure 1. The Effect of Activation of NADPH-Dependent (1) and Ascorbate-Dependent (2) Systems of Peroxide Oxidation of Microsomal Lipids on the Convergion of Cytochrome P-450 (a) to P-420 (b). System and conditions of the tests, see section "Methods of the Investigation." Content of microsomal protein in the cuvette 2.4 mg; cuvette volume 3 ml.

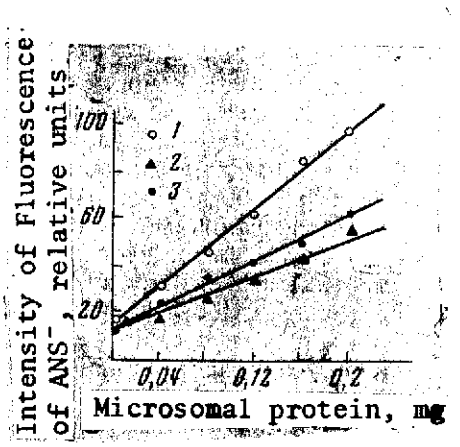


Figure 2. Intensity of Fluorescence of $ANS^-$ in a Medium Containing Control (1) Preparations of Microsomes ("zero time") and the Same Preparations Subjected to Enzymatic (2) and Nonenzymatic (3) Peroxide Oxidation of NAA Microsomal Phospholipids for a Period of 10 minutes. Primary incubation medium: 125 mM KCl + 20 mM tri-HCl, pH 7.4; length of induction wave, 360 nm; fluorescence -- 470 nm; cuvette volume 2.5 ml; $ANS^-$ -- 10 mkM

Recently it has been considered that the indicative test for membrane hydrophobity is the degree of bonding of aniline naphthalen sulfonic acid [13] with it. In our experiments a significant decrease in ANS fluorescence was shown when adding this dye to the microsomal preparations incubated for a period of 10 minutes from the moment of initiation of either of the systems of peroxide oxidation (Figure 2).

INDICES OF ACTIVITY OF REACTIONS OF POL IN MICROSOMES AND THE INFLUENCE OF EDTA AND PHENERGAN ON THESE REACTIONS
Data average of 9 runs

| Conditions of the experiment | Formation of MDA, nm/mg for 10 min. | Decrease in OP*, % | Weight of $O_2$ absorption, mkatom/mg for 1 minute |
|---|---|---|---|
| I. NADPH-dependent system | 48 | 35-40 | 0.28 |
| Same + EDTA (0.3 mM) | 0 | 0 | 0.015 |
| Same + Phenergan (5 mkM) | 0 | 0 | 0.027 |
| II. Ascorbate-dependent system | 37.5 | 28-33 | 0.22 |
| Same + EDTA (0.3 mM) | 0 | 0 | 0.015 |
| Same + Phenergan (5 mkM) | 0 | 0 | 0.014 |

*"Zero time" value taken as 100%.

A change in the spectral characteristics of cytochrome P-450 can be the result not just of peroxide oxidation of phospholipids. Such a change of cytochrome P-450 into P-420 occurs under the influence of any agents which decrease the hydrophobic reactions between components of the microsomal membrane. It is well known that deoxycholate, triton X-100 are agents which decrease the hydrophobity of lipid membranes and lead to the conversion of P-450 into P-420 [2, 3], the more so that the given effect is manifested in anaerobic conditions which exclude the possibility of formation of peroxide reaction products.

Nevertheless, the results cited above, as it seems to us, directly indicate an interrelationship between lipid peroxide oxidation and the spectral properties of the basic component of the microsomal electron transport chain and the degree of hydrophobity of the microsomal membrane.

It is vital to emphasize that in all the experiments with the initiation of peroxide oxidation reactions accompanied by the conversion of P-450 into P-420, cytochrome $b_5$ is relatively well preserved.

Hence, the findings of the experiment show that stimulating POL reactions /900
leads to a change in the hydrophobity of the microsomal membrane, and as a result of this, one observes changes in the spectral properties of cytochrome P-450.

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